Reference: *Biol. Bull.* **191**: 1–4. (August, 1996)

Contractile Connective Tissue in Crinoids

RÜDIGER BIRENHEIDE AND TATSUO MOTOKAWA

Tokyo Institute of Technology, Faculty of Bioscience & Biotechnology, Biological Laboratory, Ookayama 2-12-1, Meguro, Tokyo, 152 Japan

Active movements in animals are usually attributed to cellular protein engines, e.g., *the actin-myosin system of muscle cells. Here we report the first evidence of an extracellular contractile connective tissue, which we have found in sea lilies and feather stars (Echinodermata, Crinoida). These marine animals have arm muscles that are antagonized, not by other muscles, but by ligaments consisting of extracellular fibrils interspersed with neuron-like cell processes. Contractile cells are lacking, yet these arm ligaments actively contracted upon stimulation. The ligaments stayed in a contracted condition even after the stimulus had stopped. The stresses generated were lower than those of typical skeletal muscles. Additional data from crinoid cirri, which lack muscles entirely, corroborate the hypothesis that the connective tissue of the ligaments is contractile.*

Crinoids use their arms for filter feeding, crawling, and swimming. The arms consist of a series of ossicles interconnected by mobile joints (Figs. 1a, 2a). Muscles bend the arms orally (upwards). The aboral (downward) powerstroke has been thought to be effected by the passive elastic recoil of aboral ligaments (1); but we provide evidence here that the ligaments contract actively and thus contribute to the movements of the arms.

At rest, crinoids are anchored by a different set of appendages—the cirri (Fig. 1). Each cirrus consists of a row of ossicles joined by ligaments that lack any muscles (2, 3): yet these appendages move actively (3). We show in this study that the cirri can develop forces that are probably responsible for their movements.

Specimens of crinoids were trawled from a depth of 130 m off Numazu, Suruga Bay, Japan (sea lily *Metacrinus rotundus*) or collected in shallow waters near Kominato, Chiba Prefecture, Japan (feather star *Comanthus japonicus*). The animals were kept in circulating seawater aquaria. Pieces of arms consisting of 5 to 10 ossicles were prepared as follows: The oral half of each ossicle was removed along with its connecting ligaments and muscles; thus the remaining aboral half ossicles were connected only by their aboral ligaments (Fig. 2b). The mechanical responses—*i.e.*, displacement (Fig. 3a)—and generated forces (Fig. 3b) of these half-arm preparations were examined.

Transmission electron microscopy revealed no evidence of any muscle cells in the ligaments of the arms or cirri; these ligaments appeared to be conglomerates of collagen fibrils, microfibrils, and neuron-like cell processes (Fig. 1b). The neuron-like cell processes were distributed randomly among the fibrils; the cell bodies associated with these processes are located in the pore space of the ossicles and are connected to the nervous system (1). They are likely to be the site of the mechanism that triggered the contraction of the ligaments described below. These findings reconfirm earlier reports that the ligaments of crinoid arms (1, 4, 5, 6) and cirri (2, 3) lack muscle cells.

When arm pieces were connected to a holder at one end and a metal plate was fixed to their other end (Fig. 2b), they showed a constant slow bending under the influence of gravity. But Figure 3a shows the strong flexion against the force of gravity caused by K^+ depolarization; the ligaments shortened to less than half of their length. Contraction of the aboral ligament is the only possible means by which this flexion could have been accomplished. K^+ depolarization had no effect on samples anesthetized with seawater containing menthol, but reactivity was restored after washing in seawater. Probably the K^+ ions acted by depolarizing neural elements (7).

The possibility that the neuron-like cell processes contract upon stimulation has to be considered. Many echi-

Received 14 February 1996; accepted 29 May 1996.

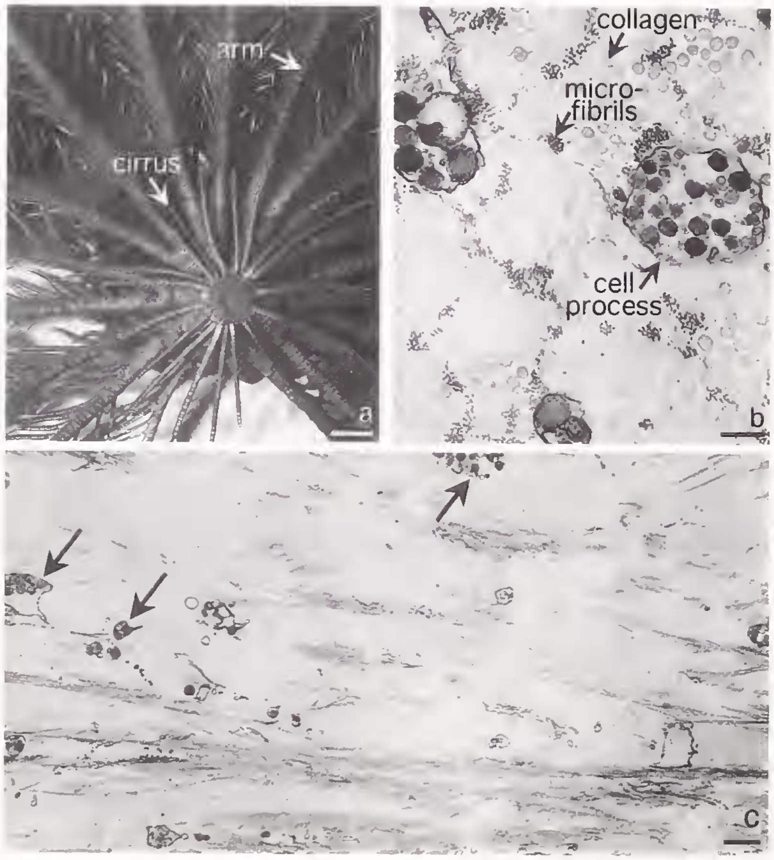

Figure 1. (a) Photograph of a feather star from the aboral side (underside). The long arms radiate from the central body and consist of a series of small ossicles connected by ligaments and muscles. Feather stars can walk or swim with their arms. The smaller cirri serve to anchor the animal when it is resting. Scale bar 5 mm. (b) Transmission electron micrograph of an aboral arm ligament of a feather star fixed after flexion of the arm and processed according to standard methods. This cross section shows that the ligament consists only of collagen, microfibrils, and neuron-like cell processes filled with dark vesicles. Scale bar 350 nm. (c) Low-power transmission electron micrograph of a longitudinal section of the aboral arm ligament of a sea lily. The ligament consists mainly of extracellular fibril bundles that run straight between two ossicles of the arm. Profiles of cell processes are distributed randomly and never run straight. Often the processes contain dark vesicles (arrows). Scale bar 1 μm.

noderm connective tissues have a structure similar to that of the crinoid ligaments and contain neuron-like cell processes (8, 9). But these processes do not seem to be specialized for contraction, and contractility has never been observed in such tissues. Moreover, the cell processes follow a tortuous path through the ligament and are connected neither to the fibrils nor the ossicles. Therefore we believe that the contractile force causing flexion of our tissue preparations can only be ascribed to the extracellular matrix with its microfibrils and collagen fibrils.

Figure 3b shows the force generated by an arm piece after K^+ depolarization. Often the force fluctuated for some time, suggesting that the force-generating mechanism is an active process, not just a passive recoil of some elastic element. In force measurements, any possibly elastic element in the ligament is subject to the same tension. If the ligament relaxes to a certain passive force (tension), every mechanical element in the ligament has relaxed to that force. In such a case, an elastic element cannot exert a force higher than that to which it has relaxed. Therefore, if a relaxation is followed by an increase of force, as shown in Figure 3b, it must be ascribed to the active generation of force. In our experiments, the ligaments eventually developed a maximal force that decreased to a lesser, constant value. The maximal force of a single ligament was calculated to be about 3 mN, corresponding to a stress of about 5.6 KPa. Skeletal muscles of vertebrates typically develop stresses of 200 KPa, two orders of magnitude higher.

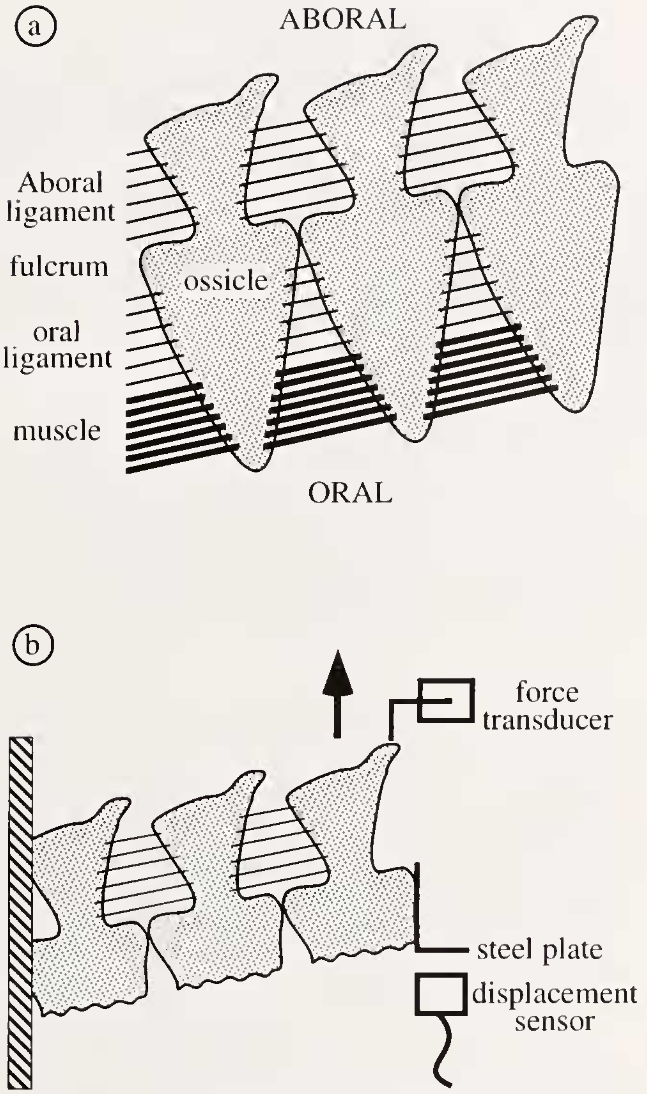

Figure 2. (a) Schematic longitudinal section of a segment of an arm of a feather star. Ossicles are movable against each other at a fulcrum, and they are connected with aboral ligaments, oral ligaments, and muscles. (b) Pieces of arm were glued to a holder oriented with the aboral side up (as in Fig. 2a). The oral part, including muscles and oral ligaments, was removed so that the ossicles were only connected *via* the aboral ligaments. Active flexion occurred in the direction of the arrow and was detected with an eddy current sensor monitoring movements of a stainless steel plate (weight about 110 mg in seawater) fixed to the free end of the preparation. In another set of experiments, forces were measured with a force-gauge whose pick-up needle was positioned on the sample. All experiments were done in a trough containing the experimental solutions at 20°C.

Contractile connective tissue stayed in the contracted state for hours even when the K^+ depolarizing solution was washed out after only 200 seconds. Probably this effect is due to the so-called "connective tissue catch," a phenomenon typical of echinoderm connective tissues (8). Such tissues can change their stiffness under nervous control and thus serve to lock extremities in given positions over a long time (10). In crinoids, such connective tissues were found in the cirri (3, 7) and in the stalk (11). The arm ligaments go one step further: they not only change passive stiffness, but also develop active contractile forces under nervous control. In summary, arms can be locked in any position by the catch mechanism of the ligaments. Movement can occur when the ligaments soften: then the arms can be moved orally by muscles, and aborally by a ligament that combines elastic properties with the ability to contract actively.

Observations on the cirri of sea lilies corroborate the hypothesis of contractile connective tissue. We have connected the proximal end of freshly dissected cirri to a

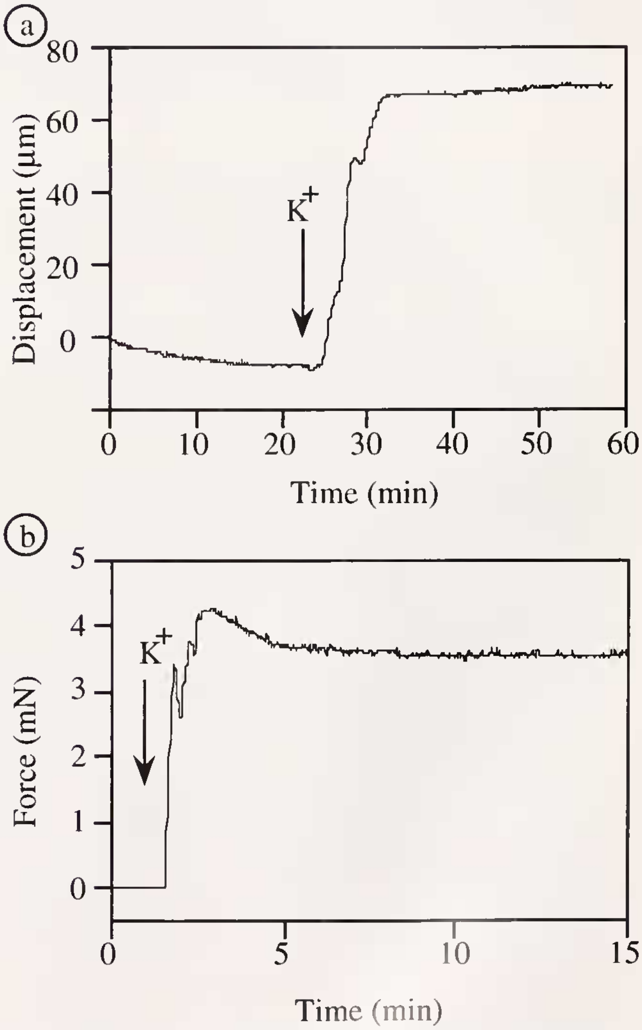

Figure 3. (a) Artificial seawater with elevated potassium concentration (100 mM) caused an upward flexion of a piece of arm of a feather star. (b) Force generated by a piece of arm of a feather star on stimulation with excess K^+.

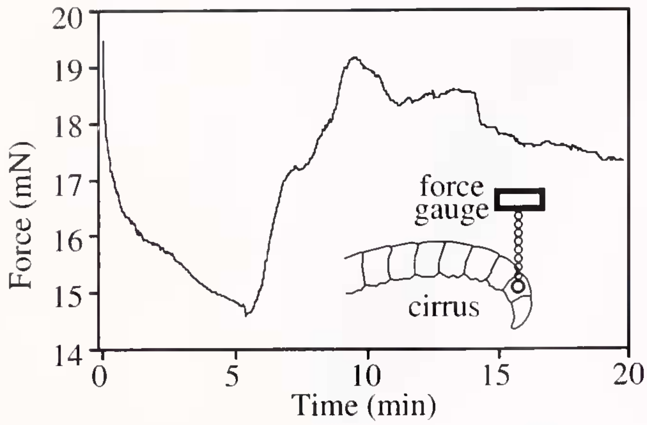

Figure 4. Freshly dissected cirri were fixed at the proximal end, and the distal end was connected *via* a silver chain to a force transducer. A microdrive connected to the force transducer caused the cirrus to bend aborally (upward). For the curve shown the cirrus was bent until the force reached about 20 mN. This position was fixed, and the cirrus was left in aerated seawater without further stimulation. A typical initial phase of stress relaxation was followed by a spontaneous production of force that fluctuated irregularly.

static holder and the distal end, *via* a clip and a chain, to a force transducer in stress-relaxation tests. We observed fluctuating forces spontaneously generated by the cirri (Fig. 4). As noted above, cirral ligaments lack any potential contractile cells, so the observed forces are probably developed by the extracellular matrix. Here, as in the arm ligaments, fluctuating forces strongly suggest an active generation of force and not a passive recoil. Thus, crinoids seem to use contractile ligaments in various parts of their body. Probably a contractile ligament is less energy-consuming than a muscle, so—despite its slow response and the comparatively low forces that it produces—it might be of adaptive significance for a filter-feeding animal.

Active contractility has been proposed for the holothurian dermis (12). But the holothurian dermis contains coelomic pouches lined by muscular tissue that might be responsible for contractions. Force-generating connective tissue in the wall of vertebrate blood vessels (which also contains muscle cells) has recently been proposed on the basis of circumstantial evidence (13). The present study provides the first unequivocal evidence of a contractile connective tissue. We believe that our findings have great importance for both animal biology and medicine.

Acknowledgments

We thank Dr. S. Kikuchi for providing feather stars and Mr. R. Ohki and Mr. T. Chiba for assistance with electron microscopy. The valuable comments of two anonymous referees helped to improve our manuscript significantly. This work was supported by a Sasakawa Research Grant of the Japan Science Society to R.B. and a grant of the Ministry of Education, Science, Sports, and Culture of Japan to T.M.

Literature Cited

1. **Birenheide, R., and T. Motokawa. 1994.** Morphological basis and mechanics of arm movement in the stalked crinoid *Metacrinus rotundus* (Echinodermata, Crinoida). *Mar. Biol.* **121:** 273–283.
2. **Holland, N. D., and J. Grimmer. 1981.** Fine structure of the cirri and a possible mechanism for their motility in stalkless crinoids (Echinodermata). *Cell Tissue Res.* **214:** 207–217.
3. **Birenheide, R., and T. Motokawa. 1995.** Motility and stiffness of cirri of the stalked crinoid *Metacrinus rotundus*. Pp. 91–94 in *Echinoderm Research 1995*. R. Emson, A. Smith, and A. Campbell, eds. A. A. Balkema, Rotterdam.
4. **Meyer, D. L. 1971.** The collagenous nature of problematical ligaments in crinoids. *Mar. Biol.* **9:** 235–241.
5. **Candia Carnevali, M. D., and A. Saita. 1985.** Muscle system organization in echinoderms: II. Microscopic anatomy and functional significance of the muscle-ligament-skeleton system in the arm of comatulids (*Antedon mediterranea*). *J. Morph.* **185:** 59–74.
6. **Welsch, U., T. Heinzeller, and J. L. S. Cobb. 1990.** Ultrastructure and innervation of ligament connective tissue in *Antedon bifida* and *Decametra* sp. Pp. 295–300 in *Echinoderm Research*. C. De Ridder, P. Dubois, M. Lahaye, and M. Jangoux, eds. A. A. Balkema, Rotterdam.
7. **Wilkie, I. C. 1983.** Nervously mediated change in the mechanical properties of the cirral ligaments of a crinoid. *Mar. Behav. Physiol.* **9:** 229–248.
8. **Motokawa, T. 1984.** Connective tissue catch in echinoderms. *Biol. Rev.* **59:** 255–270.
9. **Wilkie, I. C. 1984.** Variable tensility in echinoderm collagenous tissues: a review. *Mar. Behav. Physiol.* **11:** 1–34.
10. **Motokawa, T. 1985.** Catch connective tissue: the connective tissue with adjustable mechanical properties. Pp. 69–74 in *Echinodermata* (*Proc. 5th Int. Echinoderm Conf. Galway*). B. F. Keegan and B. D. S. O'Connor, eds. A. A. Balkema, Rotterdam.
11. **Wilkie, I. C., R. H. Emson, and C. M. Young. 1993.** Smart collagen in sea lilies. *Nature* **366:** 519–520.
12. **Jordan, H. 1914.** Über "reflexarme" Tiere. IV. Die Holothurien. *Zool. Jahrb. Jena Abt. Allg. Zool. Physiol.* **34:** 365–436.
13. **Shapovalov, A. N., I. V. Shapovalova, M. F. Shuba, V. A. Uzhvan, and V. P. Strizhak. 1994.** Connective tissue generates mechanical tension during contraction of the vascular wall. *Bull. Exp. Biol. Med.* **117:** 253–255.